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ORIGINAL ARTICLE

Nicotinism and quality of embryos obtained in *in-vitro* fertilization programmes

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Abstract

Introduction: According to the World Health Organization, infertility is defined as the inability to conceive following 12 months of regular unprotected sexual intercourse. Cigarette smoking, alcohol and drugs are the main stimulants exerting a negative effect on the male and female reproductive organs.

Objective: The objective of the study was analysis of the effect of cigarette smoking by the women examined and their partners on the quality of embryos obtained in *in vitro* fertilization programmes.

Material and methods: The study covered 54 women treated due to infertility. The database and statistical analyses were performed by means of computer software STATISTICA 7.1 (StatSoft, Poland).

Results: The study showed that among 100% of the women examined, 24.07% smoked cigarettes. No statistically significant difference was observed between cigarette smoking by the women in the study (p=0.42), and the number of cigarettes smoked daily (p=0.52) and the total duration of smoking expressed in years (p=0.56). In addition, the study showed that 33.33% of respondents were exposed to passive nicotinism, while 66.67% were not exposed to passive smoking. In the group of women exposed to passive smoking, Class A embryos constituted 11.11%, Class B embryos – 83.38%, whereas Class C embryos – only 5.56%. A statistically significant relationship was noted between classes of embryos and exposure to passive nicotinism (p=0.03). Passive smoking results in the development of embryos of poorer quality. A significantly higher number of Class 2 embryos were produced from oocytes of women exposed to the effect of cigarette smoke, compared to Class 1. Among women at reproductive age, an active campaign should be carried out against nicotinism on behalf of their fertility and future maternity.

Key words

infertility, embryos, nicotinism

INTRODUCTION

According to the World Health Organization (WHO), infertility is defined as the inability to conceive following 12 months of regular unprotected sexual intercourse. The occurrence in a woman of two consecutive spontaneous miscarriages (or stillbirths) is also considered as infertility. It is estimated that 50-80 million of the population at reproductive age worldwide are infertile (every 6th couple). In Poland, approximately 2 million males and females face fertility problems. Considering such a large scope of the phenomenon the WHO classified infertility into social diseases [1, 2].

The limited fertility of a couple or of each partner individually may lead to infertility. The female factor is responsible for approximately 45-65% of infertility cases, whereas the male factor for 24-45%, while in about 10% of cases the problem is confirmed in both partners. In the remaining cases, establishing the cause is impossible (idiopathic infertility). Cigarette smoking, alcohol, and drugs are the main stimulants exerting a negative effect on the male and female reproductive system [3, 4].

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The objective of the study was analysis of the effect of cigarette smoking by the women examined and their partners on the quality of embryos obtained in the programmes of *in vitro* fertilization.

MATERIAL AND METHOD

The presented study was conducted in the Non-Public Health Care Unit 'Ovum Reproduction and Andrology' in Lublin, and covered women treated due to infertility. The research instrument in the form of a questionnaire form was independently completed by respondents, who had been informed concerning the objective of the study and its total anonymity. A reservation was also made that the data for coding the questionnaires will be used exclusively for the identification of medical records.

A total number of 60 questionnaires were distributed, and no interferences were observed while carrying out the study. Fifty-four correctly completed questionnaire forms were qualified for statistical analysis. Women with chronic and metabolic diseases and obesity were excluded from the study group. Each questionnaire form qualified for statistical analysis was supplemented by an embryo quality sheet, for which data was collected from medical records identified, based on the codes placed on the questionnaires by respondents. Morphological assessment of the embryos was performed by means of an inverted microscope (Olympus

CKX41) with mounted digital camera (ARTCAM-500MI). At the first stage (16-20 hours after micromanipulation), an evaluation of pronuclei was performed, and unfertilized cells were rejected. After the subsequent 24 hours, embryos were evaluated, considering the properties associated with embryo's implantation capability, such as pace of division, degree of fragmentation, presence of a single nucleolus per blastomere, the same size of blastomeres and symmetry in their positioning [5, 6, 7, 8]. Embryos showing the best properties were classified into Class A, possessing the highest reproductive potential. Embryos showing slight deviations in the degree of fragmentation (10-25%), symmetry and division pace were classified into Class B. Considerable and big abnormalities in the structure of embryos were the cause for classifying them into Classes C and D, respectively. The presence of one or more single nucleolus per blastomere resulted in upgrading the embryo class by one position, while the observation of two nucleoli in one blastomere resulted in downgrading the embryo class by 2 positions.

The respondents' ages ranged between 25-39. The most numerous group constituted women aged 35-39 (44.44%, n=24), followed by women aged 30-34 (37.04%, n=20), and those aged 25-29 (n=10) – 18.52%. As many as 62.96% of the women examined were urban inhabitants (n=34), whereas 37.04% (n=20) lived in rural areas. The majority of respondents possessed university education – 66.67% of women (n=36), followed by secondary school education – 25.92% (n=14), and secondary vocational education level – 7.41% (n=4). No women in the study group had elementary education level. Women with obesity and chronic metabolic diseases were not qualified into the study group.

The results of the studies obtained were subjected to statistical analysis. The values of the parameters analyzed were determined by means of frequency and percentage. For uncorrelated nominal variables, in order to investigate differences between the classes compared, c2 goodness of fit test was applied. The relationships between the values examined were analyzed by means of the c2 test for independence. The p values $p<0.05$ were considered statistically significant. The database and statistical analysis were performed using computer software STATISTICA 7.1 (StatSoft).

RESULTS

Figure 1 presents the classes of embryos obtained from respondents during infertility treatment by the IVF-ET method. The ABCD classification reflects the quality of individual classes, where A means the best embryo, while D – the poorest quality embryo.

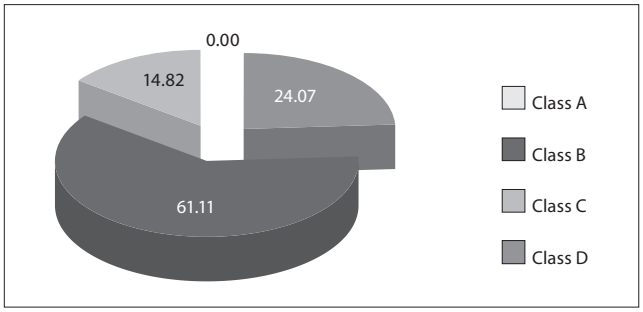

Figure 1. Distribution of respondents with consideration of classes of embryos

The studies performed indicated that the greatest number of embryos were obtained in Class B (61.11%, n=33), followed by Class A (24.07%, n=13), and Class C – 14.28% (n=8). No embryos of Class D were obtained.

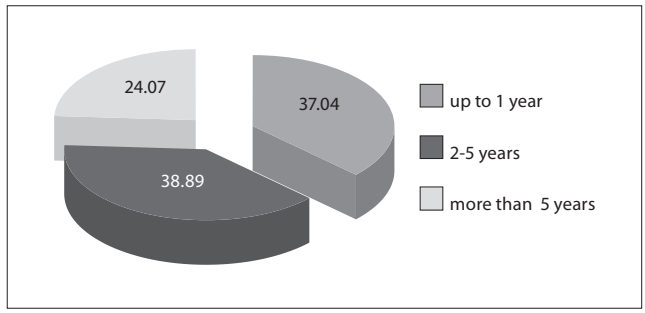

Figure 2. Distribution of respondents according to duration of infertility treatment

Figure 2 presents the structure of respondents according to the duration of infertility treatment.

Based on the results of the study, it was noted that for 37.04% of respondents (n=20) the duration of treatment was up to 1 year, while for 38.89% (n=21) of the women the period of treatment remained within the range from 2-5 years, and for 13 women (24.07%) this period was over 5 years.

Table 1 presents the distribution of embryos in individual classes, according to the period of infertility treatment in the group of women in the study. The study showed that in the group of women treated due to infertility for a period up to 1 year, Class A embryos constituted 40.00%, Class B embryos – 55.00%, whereas embryos of Class C – only 5.0%. Among women treated due to infertility for a period from 2-5 years, the percentage of Class A embryos was 19.05%, Class B – 61.90%, and Class C – 19.05%. In addition, analysis of the results indicated that in the group of patients treated for a period longer than 5 years, Class A embryos constituted 15.38%, Class B embryos – 61.54%, while embryos of Class C – only 23.08%. Among the women examined, no statistically significant difference was noted between the duration of infertility treatment and the class of embryos (p=0.31).

Table 1. Duration of infertility treatment and classes of embryos among the women examined

	Duration of treatment and class of embryo				
	Duration of treatment (intervals)	class of embryo A	class of embryo B	class of embryo C	Total
No.	up to 1 year	8	11	1	20
%		40.00%	55.00%	5.00%	100.00%
No.	2-5 years	4	13	4	21
%		19.05%	61.90%	19.05%	100.00%
No.	more than 5 years	2	8	3	13
%		15.38%	61.54%	23.08%	100.00%
No.	Total	14	32	8	54
%		25.93%	59.26%	14.81%	100.00%
$Chi^2=4.71$ p=0.31					

In addition, the study showed that among 54 (100%) women in the study, 13 (24.07%) smoked cigarettes. Among this group, the replies concerning the number of cigarettes

smoked daily were nearly equally distributed: 53.85% of women (n=7) smoked up to 5 cigarettes daily, while 46.15% (n=6) – within the range from 5-19 cigarettes daily. Considering the duration of the smoking habit, 38.46% of respondents (n=5) had smoked for 3-5 years, 38.46% (n=5) for more than 5 years, and only 23.08% of women (n=3) had smoked for a period of 1-2 years.

No statistically significant relationship was observed between cigarette smoking by the women examined (chi^2=1.70, p=0.42) and the number of cigarettes smoked daily (chi^2=1.27, p=0.52), and the total duration of smoking expressed in years (chi^2=2.97, p=0.56).

Table 2 presents a correlation between exposure to passive smoking and embryo class in the group of women in the study.

Table 2. Exposure to passive smoking and class of embryo

	Exposure to passive smoking and class of embryo				
	exposure to passive nicotinism	class of embryo A	class of embryo B	class of embryo C	Total
No.	Yes	2	15	1	18
%		11.11%	83.38%	5.56%	100.00%
No.	No	12	17	7	36
%		33.33%	47.22%	19,.44%	100.00%
No.	Total	14	32	8	54
%		25.93%	59.26%	14.81%	100.00%
Chi^2 = 6.48 p = 0.039					

The studies confirmed that 33.33% (n=18) of respondents were exposed to passive nicotinism, whereas 66.67% (n=36) were not exposed to passive smoking. In the group of women who were exposed to passive smoking, Class A embryos constituted 11.11%, Class B embryos – 83.38%, and Class C embryos – only 5.56%. Among women who were not exposed to passive smoking, Class A embryos constituted 33.33%, those of Class B – 47.22%, and Class C embryos – 19.44%. A statistically significant relationship was observed between the classes of embryos and exposure to passive smoking (chi^2=6.48, p=0.039). A significantly higher percentage of Class B embryos (83.38%) were produced from oocytes of women exposed to the effect of tobacco smoke, compared to Class A (11.11%).

DISCUSSION

Smoking, both active and passive, is hazardous for the reproductive processes. The components of cigarette smoke cause a number of changes that hinder fertilization. The changes observed in women are, among others, lower level of estrogens and progesterone, oviduct function disorder and deterioration of ovarian function (lower production of ovarian follicles). From the aspect of the egg cell, a change of its environment occurs as a result of the production of pro-inflammatory cytokines in response to smoke components, then an induction of oxidative stress, and a decrease in the number of granulocytes, which condition the adequate steroidgenesis responsible for egg cell maturation. The zona pellucida is also subject to changes – its thickening occurs, which renders the penetration of a sperm cell impossible, and after fertilization – the development of the embryo. The cytoplasm of the oozyte is dark, course grained and non-uniform. The fertilized egg cells show a delayed development, and the cleavage process is abnormal.

The results of the stimulation of ovulation are worse in smoking than non-smoking patients – smoking patients show a poorer response to the gonadotropin administration, a smaller number of follices is obtained, and the percentage of their fertilization is lower. Moreover, smoking increases the production of androgens, which are the cause of masculinization of the silhouette of smoking women.

The effect of smoking on fertility is also noted in males. A decrease is observed in the level of prolactin and testosterone, decreased volume of ejaculate, a decrease in acrosomal activity, presence of leukocytes in the semen, deterioration in the morphology of sperm and slowing down of their mobility. In studies on animals, a deterioration in the capability for implantation of embryos obtained by the IVF or ICSI methods was confirmed when the sperm was exposed to a tobacco smoke environment [3, 9, 10, 11, 12, 13].

Studies by Depy-Martynów et al. showed the deterioration of oocytes parameters and decrease in the percentage of pregnancies in women who smoked, compared to non-smokers [14]. Further studies by Depy-Martynów et al. in 2006, confirmed a statistically significant ($p<0.05$) worse quality of embryos in the group of smoking women treated due to infertility, compared to the control group [15]. Similar observations were made by Gruber et al. [16]. Based on the results of own studies, it was noted that 24.07% of the women in the study smoked cigarettes, also, 50.00% of their partners were smokers. In addition, the study confirmed that 33.33% of women were exposed to passive nicotinism. A statistically significant relationship was found between the classes of embryos and exposure to passive smoking (chi^2=6.48, p=0.039). A significantly higher percentage of Class B embryos (83.38%) developed from oocytes of women exposed to tobacco smoke, compared to Class A emryos (11.11%).

In the light of own studies, and based on relevant literature, it may be presumed that each human behaviour leaves a trace in the organism. However, attempts to unequivocally confirm this effect were successful in only one case – the consequences of exposure to passive smoking.

CONCLUSIONS

Passive smoking results in the production of embryos of poorer quality. A significantly higher percentage of Class B embryos developed from oocytes of women exposed to the effect of tobacco smoke, compared to Class A.

Among women at reproductive age, an active campaign should be carried out against nicotinism, on behalf of their fertility and future maternity.

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